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A One- and Two-Dimensional ^{13}C and ^{1}H N.M.R. Study of Some Triterpenes of the Hopane, Stictane and Flavicene Groups

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Abstract

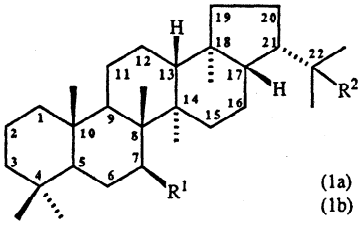
The phase sensitive two-dimensional (2D) ^{13}C–^{1}H heteronuclear correlated and double quantum filtered COSY n.m.r. spectra of the triterpenes 7β-acetoxyhopan-22-ol and 2α,3β,22α-triacetoxystictane have been recorded at a resolution sufficient for the complete assignment of their proton resonances. Revisions to some of the methylene proton resonances of 7β-acetoxyhopan-22-ol proposed elsewhere are discussed, and assignments for a series of flavicene and stictane triterpenes are presented. 2D n.m.r. analyses of a new triterpene isolated from the extracts of the lichen *Pseudocyphellaria coronata* revealed it to be 22α-hydroxystictano-25,3β-lactone.

Introduction

In connection with studies directed towards the structural elucidation of some new hopane and stictane triterpenoids isolated from lichen sources, we had occasion to examine the ^{13}C and ^{1}H n.m.r. spectra of a number of hopane, stictane and flavicene triterpenoids of established structure.[1–3] Anomalies in the ^{13}C n.m.r. assignments proposed elsewhere for hopane (1a)[4,5] and for 7β-acetoxyhopan-22-ol (1b)[6] prompted our recent investigations of the ^{13}C n.m.r. spectra of some epimeric hydrocarbons of the hopane group,[7] and of some oxygenated analogues.[8] While the latter study resulted in revisions to many of the ^{13}C and ^{1}H n.m.r. methyl group and methine proton assignments proposed for 7β-acetoxyhopan-22-ol (1b), it did not address the anomalies which were apparent in the methylene proton assignments deduced[6] from an analysis of n.O.e. difference spectra.

[1] Corbett, R. E., and Young, H., *J. Chem. Soc. C*, 1966, 1556, 1564,
[2] Chin, W. J., Corbett, R. E., Heng, C. K., and Wilkins, A. L., *J. Chem. Soc., Perkin Trans. 1*, 1973, 1437.
[3] Corbett, R. E., and Wilkins, A. L., *J. Chem. Soc., Perkin Trans. 1*, 1976, 857, 1316.
[4] Balogh, B., Wilson, D. M., Christiansen, P., and Burlingame, A. L., *Nature*, 1973, **242**, 603.
[5] Wenkert, E., Baddeley, G. V., Burfitt, I. R., and Moreno, L. N., *Org. Magn. Reson.*, 1978, **11**, 337.
[6] Howarth, O. W., Richard, T. M. A., and Sainsbury, M., *Org. Magn. Reson.*, 1983, **21**, 56.
[7] Wilkins, A. L., Bird, P. W., and Jager, P. M., *Magn. Reson. Chem*, 1987, **25**, 503.
[8] Wilkins, A. L., Bird, P. W., Jager, P. M., and Ronaldson, K. J., *Aust. J. Chem.*, 1987, **40**, 1713.

A major disadvantage of the n.O.e. difference technique is that irradiation at a chosen frequency invariably results in enhancement of the resonances of several nearby protons, with a consequent uncertainty as to the resonance of an individual proton, a situation which is further compounded by the fact that even at high field several protons resonate at the same frequency. However, this uncertainty can be overcome in two-dimensional (2D) n.m.r. experiments in which ^{1}H and ^{13}C n.m.r. signals are uniquely correlated. In this paper we report the application of 2D n.m.r. experiments which lead to a complete assignment of the proton resonances of 7β-acetoxyhopan-22-ol (1b) and 2α,3β,22α-triacetoxystictane (2q), and the characterization of a new triterpene lactone.



	R^1	R^2
(1a)	H	H
(1b)	OAc	OH

2D N.M.R. Study of 7β-Acetoxyhopan-22-ol

A variety of 2D n.m.r. experiments can be employed[9,10] to establish the ^{1}H and ^{13}C n.m.r. resonances of large organic molecules. The improved line shape of phase sensitive procedures, in comparison to that of absolute value procedures, greatly assists the analysis of fully coupled 2D n.m.r. spectra, and in consequence a complete assignment of the proton resonances of a triterpenoid can be achieved. The fully coupled phase sensitive heteronuclear ^{13}C–^{1}H correlated (PSHCOR) n.m.r. spectrum of 7β-acetoxyhopan-22-ol (1b) is presented in Fig. 1. The inclusion in the PSHCOR sequence of a birotational decoupler[11] affords a partially decoupled 2D ^{13}C–^{1}H correlated n.m.r. spectrum, in which only ^{2}J couplings are retained. In circumstances where the ^{13}C n.m.r. assignments are known the ^{1}H n.m.r. resonances of directly bonded protons can be determined from an analysis of the cross peaks which appear in the fully coupled PSHCOR spectrum, provided the digital resolution of the 2D n.m.r. data set is such that $^{2}J_{(H\text{–}C\text{–}H)}$ and $^{3}J_{(H\text{–}C\text{–}C\text{–}H)}$ axial–axial couplings of the order 10–14 Hz can be distinguished from $^{3}J_{(H\text{–}C\text{–}C\text{–}H)}$ equatorial–equatorial or equatorial-axial couplings of the order 3–5 Hz.

The stereochemical assignment of the methylene protons attached to C1, C3, C15 and C19 serves to illustrate the interpretation of the cross peaks. The ^{13}C n.m.r. assignments of these carbons are well established,[8] and occur at 40·1, 41·8, 36·6 and 41·4 ppm respectively. H1α and H3α are coupled with their respective β-face protons ($^{2}J = 14·0$ and 13·8 Hz respectively), and to H2β [$^{3}J_{(ax\text{–}ax)} \approx 10$ Hz] and H2α [$^{3}J_{(ax\text{–}eq)} \approx 4$ Hz]. The resolution of the 2D n.m.r.

[9] Morris, G. A., *Magn. Reson. Chem.*, 1986, **24**, 371.
[10] Derome, A. E., 'Modern NMR Techniques for Chemistry Research' (Pergamon: Oxford 1987).
[11] Garbow, J. R., Weitekamp, D. P., and Pines, A., *Chem. Phys. Lett.*, 1982, **93**, 504.

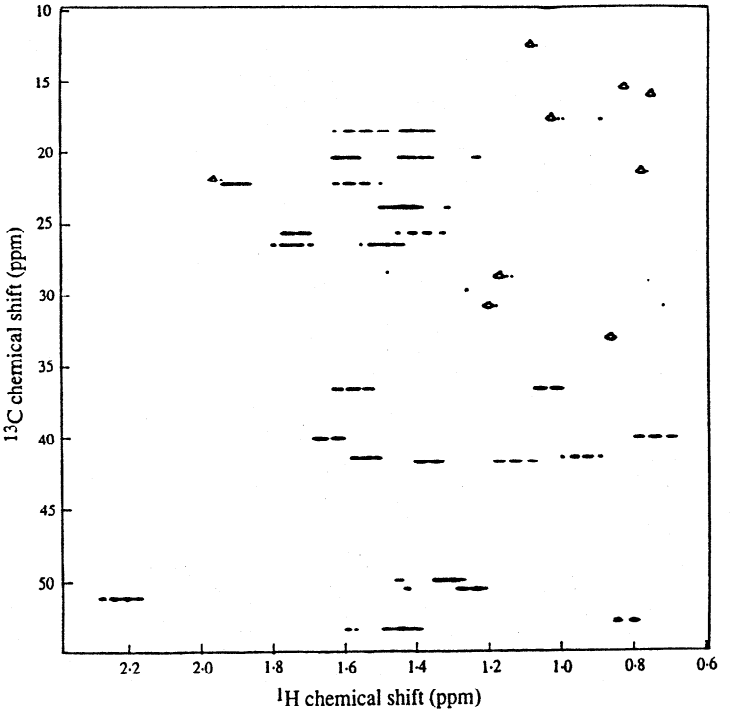


Fig. 1. Coupled two-dimensional phase sensitive heteronuclear ^{13}C–^{1}H correlated (PSHCOR) n.m.r. spectrum of 7β-acetoxyhopan-22-ol (1b).

data set is such that only the larger of these couplings are adequately resolved, hence H1α and H3α give rise to triplet-like signals. On the other hand H1β and H3β are coupled ($^{2}J = 14 \cdot 0$ and $13 \cdot 8$ Hz respectively) with their respective α-face protons, and to H2α [$^{3}J_{(eq-eq)} \approx 4$ Hz] and H2β [$^{3}J_{(eq-ax)} \approx 4$ Hz]. Since only the larger ^{2}J coupling is adequately resolved H1β and H3β appear as broadened doublets. The multiplicity of these signals is apparent in a contour plot (Fig. 1) and in cross sections profiles (lodged as supplementary material; see Deposited Material under Experimental). It is thus apparent that H1α and H3α, both of which are oriented 1,2-*trans* with respect to an adjacent axial methyl group, resonate at comparatively high field ($0 \cdot 73$ and $1 \cdot 13$ ppm respectively), whereas the equatorial H1β and H3β signals occur at significantly lower field ($1 \cdot 63$ and $1 \cdot 35$ ppm respectively). The upfield shifts experienced by H1α and H3α in (1b) can be compared with those experienced by H1α and H12α in steroids.[12] Since H15β is oriented 1,2-*trans* with respect to the 14α-methyl group, it could have been expected that H15β would occur at higher field than H15α. However, multiplicity considerations require that H15α (doublet-like signal) and H15β (triplet-like signal) resonate at $1 \cdot 02$ and $1 \cdot 57$ ppm respectively. The nearby 7β-acetate group may be responsible for the increased shielding of H15α and the deshielding of H15β.

The doublet of doublet and quartet-like patterns exhibited by C19 ($41 \cdot 4$ ppm) distinguish this methylene carbon of the five-membered ring from the

[12] Hall, L. D., and Sanders, J. K. M., *J. Org. Chem.*, 1981, **46**, 1132.

other methylene carbons of the six-membered ring. The geminal coupling exhibited by H19α and H19β ($^2J = 11 \cdot 1$ Hz) is significantly smaller than that determined for the other methylene groups of 7β-acetoxyhopan-22-ol (1b), and can be compared with the similarly small geminal coupling displayed by the C15 methylene protons in steroids.[13] The latter five-membered ring carbon is, like C19 of 7β-acetoxyhopan-22-ol (1b), adjacent to an axial bridgehead methyl group.

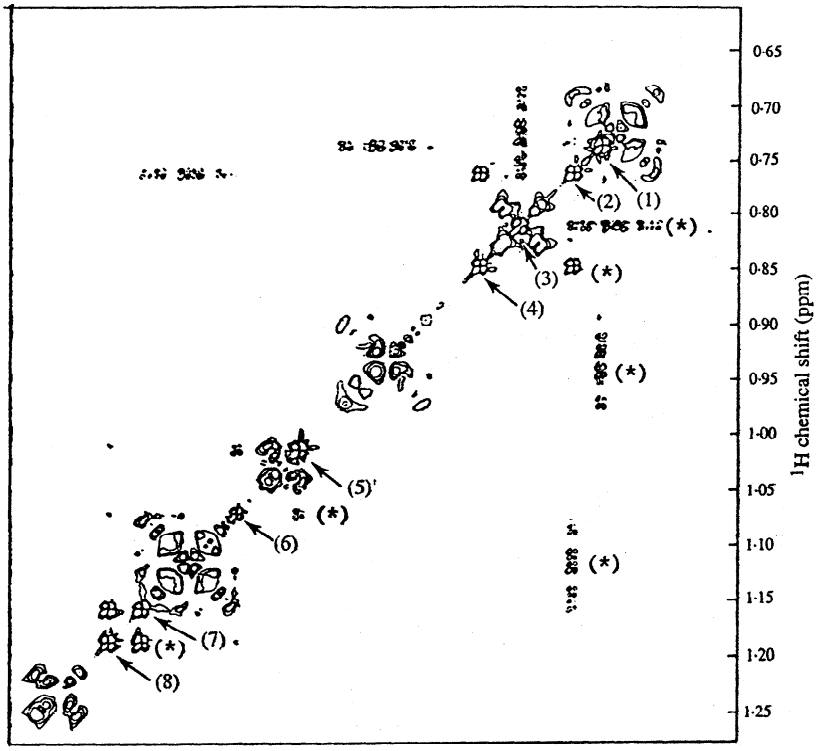


Fig. 2. Symmetrized phase sensitive double quantum filtered COSY (PSDQFC) n.m.r. spectrum (expansion of the region 0·6–1·3 ppm) determined for 7β-acetoxyhopan-22-ol (1b). Diagonal peaks from the protons of the 18α (1), 4β (2), 10β (3), 4α (4), 14α (5), 8β (6) and C22 (7) and (8) methyl groups are arrowed, while ^4J and/or ^5J coupled methyl–methyl and methyl–methylene proton cross peaks are designated by asterisks. Signal assignments are given in Table 1.

The cross peaks which occur in the phase sensitive double quantum filtered COSY (PSDQFC) spectrum of 7β-acetoxyhopan-22-ol (1b) (a portion of which is presented in Fig. 2) afford additional support for the ^{1}H n.m.r. methyl group assignments presented in our earlier study.[8] A noteworthy aspect of the PSDQFC spectrum is that the quaternary methyl group signals, while traditionally regarded as uncoupled singlet signals, do in fact experience long range ^4J or ^5J couplings. For example the methyl group signals which resonate at 0·73, 0·77 and 0·81 ppm displayed cross peaks centred at 0·93 (H19α), 1·13 (H3α) and 0·73 (H1α) ppm respectively. These ^4J coupled protons

[13] Hall, L. D., and Sanders, J. K. M., *J. Am. Chem. Soc.*, 1980, **102**, 5703.

are oriented 1,2-*trans* with respect to the 18β-, 4β- and 10β-methyl groups respectively. A ^4J coupling also exists between the protons of the 4α- and 4β-methyl groups, and the two C22 methyl groups.

As in the steroid skeleton,[14] where more than one proton is axially oriented with respect to an adjacent methyl group (e.g. H1α and H9α are similarly oriented with respect to the 10β-methyl group), ^4J coupling is observed only with 1,2-*trans* oriented methylene protons, but not with similarly oriented methine protons. On this basis it can be reasoned that the 14α-methyl group is likely to be ^4J coupled with H15β, whereas the 8β-methyl group signal is not likely to be broadened by ^4J coupling with H7α. Hence in a conventional ^{1}H n.m.r. spectrum the line width of the 8β-methyl group ($W_{h/2} = 1 \cdot 4$ Hz) is less than that of the 14α-methyl group ($W_{h/2} = 1 \cdot 9$ Hz). Although devoid of a ^4J coupling, the 8β-methyl group protons are weakly ^5J coupled with the 14α-methyl group protons (see Fig. 2). Amongst the methyl signals of the acetate (1b) only the acetoxy methyl group did not afford a diagonal peak in the PSDQFC n.m.r. spectrum. The magnitude of the ^4J and/or ^5J couplings experienced by the other methyl groups can be estimated from the magnitude of the diagonal and cross peaks which appear in the PSDQFC spectrum. In this respect the double quantum filtered COSY experiment (especially the phase sensitive variant) is superior to the conventional COSY experiment, since in the former the presence of a diagonal peak demonstrates the existence of long range coupling, whereas in a conventional COSY experiment diagonal peaks are retained irrespective of whether or not long coupling exists. Other workers[9] have noted the ability of COSY and double quantum filtered COSY experiments to detect couplings of about one-fifth the natural line width of a conventional n.m.r. signal.

Table 1. ^{13}C and ^{1}H n.m.r. assignments and ^2J coupling constants determined for the methylene carbons of 7β-acetoxyhopan-22-ol (1b)

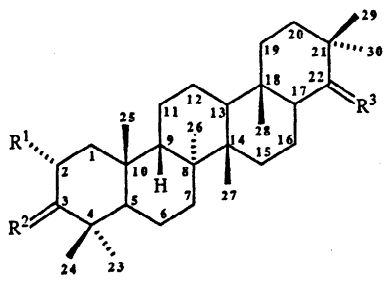
	δ ^{13}C	δ ^{1}Hα	δ^1Hβ	$^2J_{(H-C-H)}$ (Hz)
C1	40·1	0·73 (*ax*)	1·63 (*eq*)	14·0[A]
C2	18·6	1·38 (*eq*)	1·55 (*ax*)	14·8
C3	41·8	1·13 (*ax*)	1·35 (*eq*)	13·8
C6	25·7	1·72 (*eq*)	1·37 (*ax*)	13·3
C11	20·4	1·36 (*eq*)	1·57 (*ax*)	12·7
C12	23·9	1·42 (*ax*)	1·42 (*eq*)	[B]
C15	36·6	1·02 (*eq*)	1·57 (*ax*)	12·9
C16	22·3	1·55 (*ax*)	1·89 (*eq*)	12·2
C19	41·4	1·53 (*eq*)	0·93 (*ax*)	11·1
C20	26·5	1·73 (*ax*)	1·47 (*eq*)	13·0

[A] ±0·4 Hz, sign of coupling not determined but presumably negative.
[B] AA′ multiplet, ^2J not resolved.

Table 1 lists the methylene proton assignments established for 7β-acetoxyhopan–22-ol (1b); methyl and methine ^{1}H and ^{13}C n.m.r. assignments are as reported previously[8] and are not repeated here. A complete tabulation of the ^{13}C and ^{1}H n.m.r. chemical shifts and T_1 values of (1b) has been lodged as supplementary material (see Deposited Material under Experimental).

[14] Marat, K., Templeton, J. F., and Sashi Kumar, V. P., *Magn. Reson. Chem.*, 1987, **25**, 25.

When attempting to establish the C21 configuration of the 7β-acetate (1b) by means of n.O.e. difference experiments, Howarth *et al.*[6] observed that an enhancement occurred at 1·37 ppm on irradiation at 1·85 ppm. They considered these signals as arising from H17β and H21β respectively and concluded that the C21 side chain was therefore α-oriented. Our study establishes that the ^{1}H n.m.r. signal that occurs at 1·89 ppm arises from H16β. In the original study[6] nuclear Overhauser effects appear to have been interpreted largely in terms of 1,3-diaxial methyl–methyl, methyl–proton or proton–proton interactions. However, analogous studies in steroids[12] and triterpenes[15] have found that 1,2-diequatorial or axial–equatorial proton–proton or proton–methyl interactions also give rise to nuclear Overhauser effects. With this proviso some of the previously reported results can be reinterpreted in support of the assignments presented here. For example Howarth *et al.*[6] observed that irradiation of the 4β-methyl group signal (0·81 ppm) caused 'changes' (i.e. enhancements) in the vicinity of 1·16 ppm; hence H2β was considered to resonate at this frequency. Amongst the ring A methylene protons only H3β (1·13 ppm in our study) occurs in the vicinity of 1·16 ppm. As in the lupane skeleton[15] this signal should experience an n.O.e. on irradiation of the 4β-methyl group. Consistent with this assignment Howarth *et al.*[6] observed that on irradiation at 0·81 ppm, the resonance frequency of H5α and the 10β-methyl group,[8] enhancements were observed at 1·23, 1·37 and 1·52 ppm. We attribute these signals to H9α, H6β, and H2β (1·23, 1·37 and 1·55 ppm in our study) respectively.



	R^1	R^2	R^3
(2a)	H	H_2	H_2
(2b)	H	H_2	O
(2c)	H	H,β-OH	H,α-OH
(2d)	H	H,β-OAc	H,α-OAc
(2e)	H	H,β-OH	H,β-OH
(2f)	H	H,β-OAc	H,β-OAc
(2g)	H	O	H,α-OH
(2h)	H	O	O
(2i)	OH	H,β-OH	H,α-OAc

	R^1	R^2	R^3
(2j)	OAc	H,β-OAc	H,α-OH
(2k)	OAc	H,β-OH	H,α-OH
(2l)	OH	H,β-OAc	H,α-OH
(2m)	OH	H,β-OH	H,α-OH
(2n)	OAc	O	O
(2o)	OAc	H,β-OAc	O
(2p)	OAc	H,β-OAc	H,β-OH
(2q)	OAc	H,β-OAc	H,α-OAc

Stictane Triterpenes

The predictive approach of Beierbeck *et al.*[16] has general application to triterpenes and, in the case of stictane (2a), proved useful (see Table 2) in the initial assignment of the majority of the carbon signals of this pentacyclic hydrocarbon, other than those of carbon atoms in the vicinity of the boat ring B system. Thereafter substituent group effects served to distinguish individual carbon resonances. For example in stictane (2a) the chemical shifts of the C1, C7, C19 and C20 triplet carbons (34·6, 34·5, 34·8 and 36·0 ppm

[15] Preiss, A., Lischewski, M., and Adam, G., *Magn. Reson. Chem.*, 1986, **24**, 915.
[16] Beierbeck, H., Saunders, J. K., and ApSimon, J. W., *Can. J. Chem.*, 1977, **55**, 2813.

Table 2. ^{13}C n.m.r. chemical shifts of some stictane triterpenes (2a–i) and (10)

	(2a)		(2b)	(10)	(2c)	(2d)	(2e)	(2f)	(2g)	(2h)	(2i)
C1	34·6[A]	(35·0)[B]	34·4	23·9	33·2	32·8	33·2	33·0	31·7	31·6	41·4 (–14)[C]
C2	20·2	(17·6)	20·2	25·7	29·2	25·3	29·2	25·3	33·9	33·8	70·8 (420)
C3	41·5	(42·5)	41·5	82·5	79·3	81·1	79·3	81·3	220·5	220·1	84·3 (404)
C4	33·7	(33·9)	33·8	38·3	39·2	38·2	39·2	38·2	47·0	47·2	38·7 (–16)
C5	49·1	(47·2)	49·2	33·4	48·0	48·0	48·0	48·0	43·4	43·3	47·9 (51)
C6	19·5	(17·6)	19·4	20·2	19·1	18·9	19·1	18·9	20·6	20·6	18·8 (18)
C7	34·5	(30·4)	34·4	32·7	34·5	34·3	34·6	34·4	33·9	33·8	34·3 (13)
C8	42·0	(43·2)	42·1	40·3	41·9	41·8	41·9	41·9	42·0	42·0	41·7 (24)
C9	46·0	(44·4)	45·9	46·7	45·8	45·8	46·2	45·9	47·5	47·4	46·1 (24)
C10	37·2	(37·6)	37·2	44·9	36·9	36·8	36·9	36·8	36·1	36·2	37·4 (31)
C11	22·7	(22·2)	22·6	20·8	22·6	22·6	22·5	22·6	22·1	22·0	22·7 (25)
C12	21·8	(22·2)	22·2	21·7	21·5	21·5	21·3	21·4	21·3	22·2	21·4 (14)
C13	48·6	(47·2)	48·6	48·5	48·6	48·8	49·8	49·6	48·6	48·6	48·7 (12)
C14	43·3	(41·3)	43·5	42·8	42·8	42·5	43·4	43·3	42·3	43·3	42·5 (13)
C15	32·1	(33·2)	30·4	31·6	31·8	31·6	32·6	32·3	31·7	30·4	31·4 (11)
C16	25·9	(26·7)	17·7	19·2	19·4	19·6	23·5	22·8	19·3	17·6	19·6 (6)
C17	42·8	(41·6)	55·1	49·0	49·0	46·6	45·9	45·6	48·9	55·0	46·5 (8)
C18	36·6	(37·6)	42·5	38·5	38·5	38·8	36·5	36·5	38·6	42·2	38·9 (9)
C19	34·8[A]	(33·2)	35·7	34·9	34·9[A]	34·9	35·6	35·4	34·8	35·6	34·8 (8)
C20	36·0	(37·7)	36·9	35·0	35·0[A]	34·9	30·2	31·0	35·0	36·8	34·8 (8)
C21	30·6	(30·2)	43·8	35·8	35·8	35·3	34·8	34·1	35·8	43·8	35·2 (2)
C22	42·1	(42·5)	217·7	76·5	76·5	78·5	81·3	81·1	76·5	217·3	78·4 (6)
C23	33·9	(34·2)	33·9	26·4	29·1	29·0	29·1	29·0	29·4	29·3	29·6 (53)
C24	22·0	(23·7)	22·0	20·2	16·1	17·1	16·1	17·2	19·6	19·6	17·2 (51)
C25	23·1	(15·6)	23·1	178·2	22·7	22·7	22·7	22·8	23·4	23·3	23·8 (42)
C26	22·6	(13·0)	22·5	22·1	22·7	22·6	22·5	22·8	22·1	22·0	22·5 (18)
C27	17·2	(19·1)	16·8	17·1	17·3	17·1	17·3	17·2	17·2	16·5	17·1 (10)
C28	12·1	(14·6)	13·7	13·5	13·5	13·5	15·9	15·3	13·6	13·7	13·4 (7)
C29	33·5	(34·7)	25·6	29·8	29·9	29·5	27·8	27·4	29·9	25·5	29·3 (6)
C30	23·0	(25·6)	26·5	18·5	18·6	19·6	26·0	25·8	18·5	26·4	19·6 (3)
$OCOCH_3$						21·3		21·4			20·9(7)
$OCOCH_3$						21·0		21·0			
$OCOCH_3$						171·1		170·9			171·0 (6)
$OCOCH_3$						171·1		171·1			

[A] Assignments within a column of the same multiplicity may be interchanged.
[B] Calculated chemical shifts after Beierbeck *et al.*[16] are given in parentheses.
[C] Lanthanide induced shifts in Hz for $Eu(dpm)_3$ appear in parentheses.

respectively) are not reliably differentiated by their calculated shifts (Table 2). However, the introduction of a 22-keto group shields C19 and C20, but not C1 or C7, by *c.* 1 ppm. Of the last two carbons C1, but not C7, is sensitive to substitution at C3 and at C2. In a like manner the assignments of the C5 and C13 doublet carbon signals of stictane (2a) (49·1 and 48·6 ppm), while not distinguished by their calculated values (47·2 ppm in both cases), are distinguished by the LIS data presented [for (2i)] in Table 2. In the dihydroxy acetate (2i) the doublet carbon signal which occurs at 47·9 ppm must clearly be assigned to C5, since this carbon is closer to the C2 and C3 oxygen functions than is C13 (48·7 ppm). An analogous assignment (48·6 ppm) can therefore be made for C13 of stictane (2a), hence the C5 resonance of (2a) occurs at 49·1 ppm.

The marked shielding of C16 by a 22-keto group in (2b,h,n,o) can be compared to the analogous[17] shielding of the structurally equivalent C4 atom of 6-keto steroids, or of C6 in 4-keto steroids, and, in terms of the predictive approach of Beierbeck *et al.*,[16] can be correlated with the elimination of 1,3-diaxial interactions such as the H16β–H22β interaction which occurs in stictane (2a), but not in the 22-keto derivatives. Similarly the presence of a

[17] Blunt, J. W., and Stothers, J. B., *Org. Magn. Reson.*, 1978, **11**, 163.

Table 3. ^{13}C n.m.r. chemical shifts of some stictane, flavicene and hopene triterpenes (2j–p) and (3)–(6)

	(2j)	(2k)	(2l)	(2m)	(2n)	(2o)	(2p)	(3)	(5)[A]	(4)	(6)[A]
C1	39·1	39·0	43·0	41·6	40·5	39·1	39·1	39·2	40·4	39·0	40·4
C2	72·0	75·1	69·7	71·2	73·6	72·0	71·9	71·9	18·8	71·8	18·7
C3	81·1	81·3	85·6	84·7	210·0	81·2	81·2	81·0	42·2	81·8	42·1
C4	39·0	39·4	39·1	39·1	48·2	39·1	39·1	39·1	33·3	39·0	33·4
C5	47·6	47·8	47·9	48·0	48·4	47·7	47·6	47·6	56·2	47·6	56·3
C6	18·8	19·0	19·0	19·0	19·7	18·8	18·8	18·9	18·8	18·7	18·7
C7	34·0	34·2	34·1	34·3	33·6	34·1	34·1	34·4	33·4	34·4	33·4
C8	41·7	41·8	41·9	41·9	42·0	41·9	41·8	41·6	42·3	41·4	41·9
C9	46·2	46·2	46·4	46·5	45·6	46·2	46·3	46·5	50·5	46·6	50·9
C10	37·4	37·5	37·5	37·7	36·9	37·5	37·4	37·5	37·5	37·5	37·5
C11	22·7	22·7	22·8	22·9	22·7	22·8	22·7	22·7	21·0	22·8	21·3
C12	21·3	21·4	21·5	21·6	21·9	22·0	21·0	24·1	24·1	24·0	24·1
C13	48·5	48·8	48·9	48·9	48·4	48·4	49·6	48·9	48·8	49·4	49·3
C14	42·5	42·5	42·6	42·6	43·3	43·4	43·4	43·3	42·0	43·0	41·9
C15	31·7	31·7	31·8	31·9	30·3	30·4	32·5	32·7	32·7	31·8	31·8
C16	19·3	19·3	19·4	19·4	17·5	17·6	23·4	20·6	21·0	19·5	19·1
C17	48·8	48·5	48·6	48·7	54·8	55·0	46·0	54·2	54·0	139·7	140·0
C18	38·4	38·4	38·5	38·5	42·4	42·5	36·4	44·2	44·3	49·8	49·8
C19	34·8	34·8	35·1	35·1	35·6	35·7	35·6	40·0	40·3	41·4	41·6
C20	34·9	34·9	35·0	35·1	36·8	36·9	30·1	27·4	27·4	27·4	27·5
C21	35·7	35·7	35·8	35·9	43·7	43·8	34·8	47·9	48·0	136·2	136·0
C22	76·5	76·3	76·5	76·6	217·2	217·3	81·2	148·1	148·3	26·3	26·4
C23	29·4	29·5	29·5	29·8	26·8	29·5	29·4	29·4	33·5	29·3	33·4
C24	17·9	17·0	18·0	17·3	21·1	18·0	17·9	18·0	21·7	17·9	21·6
C25	23·7	23·6	23·9	24·0	23·3	23·7	23·7	23·7	16·0	23·6	16·2
C26	22·4	22·4	22·6	22·6	22·1	22·4	22·6	22·6	16·7	22·5	16·4
C27	17·2	17·2	17·3	17·2	16·7	16·8	17·1	17·1	16·9	15·0	15·0
C28	13·4	13·5	13·6	13·6	13·6	13·7	15·9	15·1	15·2	18·7	19·1
C29	29·8	29·8	29·9	29·8	26·4	26·5	27·7	109·3	109·5	21·2	21·3
C30	18·5	18·6	18·6	18·6	25·4	25·5	26·5	19·6	19·7	21·8	21·9
$OCOCH_3$	20·9	21·4	21·2		20·8	20·9	20·9	20·9		20·9	
$OCOCH_3$	20·9					21·2	20·9	20·9		20·9	
$OCOCH_3$	170·9	171·5	172·5		170·0	170·5	170·5	170·9		170·9	
$OCOCH_3$	170·9					171·0	170·9	170·9		170·9	

[A] Assignments taken from ref. 7.

22α-substituent eliminates the H16α–H22α interaction, while the presence of a 22β-substituent eliminates the H16β–H22β and H20β–H22β interactions; hence upfield shifts are experienced by C16 in (2c,d,g) and (2i–m) and by C20 in (2e,f,p) (see Tables 2 and 3).

In the stictane skeleton the boat ring B system leads to C26 (22·6 ppm) experiencing a flagpole interaction with H5α. This interaction appears to be principally responsible for the shielding of C26 by *c.* 6–7 ppm more than is typically the case for axial methyl group carbons in other chair ring B triterpenes.[5,7,16] We find that in stictane (2a) the chemical shifts of C25 and C26 differ substantially from those calculated after Beierbeck *et al.*,[16] assuming only chair-type interactions. Clearly additional boat ring parameters are required if the chemical shifts of these carbons are to be reliably calculated.

Assignments proposed for the 3β-substituted stictanes (2c–f) follow directly from those established for stictane (2a) and, in the case of the ring A carbons, correspond closely to those reported[5,18] for some 3β-substituted lupane triterpenoids. On the other hand it is apparent that for the 3-keto stictanes (2g,h) the substituent group increments attributable to the 3-keto

[18] Corbett, R. E., Cong, A. N. T., Thomson, R. A., and Wilkins, A. L., *J. Chem. Soc., Perkin Trans. 1*, 1985, 2051.

group differ significantly from those associated with the introduction of a 3-keto group into the lupane[18] or hopane[8] skeletons. This apparent discrepancy is in accord with the view[3] that in the solution state, as recently demonstrated[19] in the solid state, rings A and B of 3-keto stictanes adopt a double boat conformation.

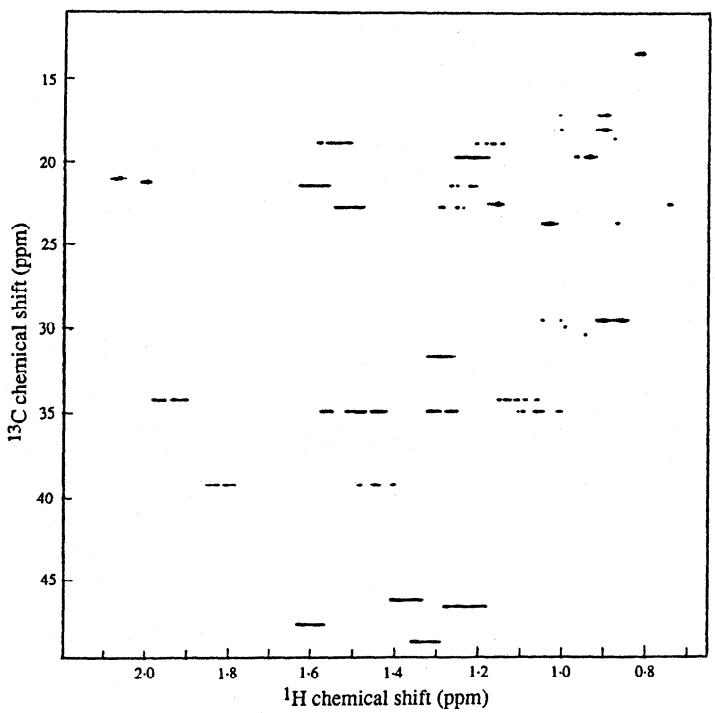


Fig. 3. Coupled two-dimensional phase sensitive ^{13}C–^{1}H heteronuclear correlated (PSHCOR) n.m.r. spectrum of 2α,3β,22α-triacetoxystictane (2q).

2D N.M.R. Study of 2α,3β,22α-Triacetoxystictane

The proton resonances of 2α,3β,22α-triacetoxystictane (2q) (see Table 4) were established in a like manner to that described for 7β-acetoxyhopan-22-ol. The proton multiplicities observed in cross section profiles taken from the fully coupled PSHCOR spectrum of the triacetate (2q), the contour plot of which is presented as Fig. 3, were in all cases in accord with the ^{2}J and ^{3}J couplings attributable to individual protons. Where unresolved multiplets were observed, the multiplet of greater half band width can be attributed to the proton which experiences the greater number of $^{3}J_{(ax-ax)}$ couplings. In the case of the twist boat ring B protons, the dihedral angles were available[20] from an X-ray crystallographic study of stictane-3β,22α-diol (2c). Additional support for the assignments presented in Table 4 was obtained in a PSDQFC experiment. Amongst the array of diagonal and cross peaks observed for (2q) were those arising from ^{4}J couplings between the protons of the 10β-, 8α-, 18β- and

[19] Wilkins, A. L., and Goh, E. M., *Aust. J. Chem.*, 1988, **41**, 143.
[20] Corbett, R. E., Simpson, J., Goh, E. M., Nicholson, B. K., Wilkins, A. L., and Robinson, W. T., *J. Chem. Soc., Perkin Trans. 2*, 1982, 1339.

21α-methyl groups (1·03, 1·14, 0·80 and 0·93 ppm respectively) with H1α, H7β, H19α and H20β (1·44, 1·93, 1·05 and 1·56 ppm respectively). Each of these protons is oriented 1,2-*trans* with respect to an adjacent methyl group and, as in the hopane skeleton, only axially oriented methylene protons (as opposed to similarly oriented methine protons) were found to exhibit ^4J couplings. The 21α- and 21β-methyl groups (0·93 and 0·84 ppm) also exhibited a cross peak indicative of a mutual ^4J coupling.

Hopane and Flavicene Triterpenes

Since an antipodal relationship exists between rings C/D/E of 21αH-hop-22(29)-ene and flavic-22(29)-ene triterpenes (Fig. 4), and between hop-17(21)-ene (6) and flavic-17(21)-ene triterpenes, the chemical shifts of their ring C/D/E carbon atoms are comparable (Table 3). Amongst these carbon atoms of the flavicenes (3) and (4) only those in the immediate vicinity of the boat ring B system display shifts which differ by more than ±0·3 ppm from those reported[7] for the corresponding carbon atoms of 21α-hop-22(29)-ene (5) and hop-17(21)-ene (6) respectively. It is noteworthy that while there is a close correspondence in the C30 signals of 2α,3β-diacetoxyflavic-22(29)-ene (3) (19·6 ppm) and 21α-hop-22(29)-ene (5) (19·7 ppm), the C30 signal of the hop-22(29)-ene occurs[7] at 25·0 ppm. These observations confirm our proposal[2] that dehydration and ring contraction of 22α-hydroxylated stictane triterpenoids take place with the extrusion of an α-face (rather than a β-face) isopropenyl side chain.

T_1 Data

Comparatively little information is presently available in respect of the structural signficance of ^{13}C T_1 values in triterpenoids, other than for triterpenes of the friedelane group.[21] The latter studies have established that amongst the axial methyl group carbons, the more highly hindered methyl carbons display shorter T_1 values most probably because of a decreased spin–rotation contribution to relaxation. The methyl group ^{13}C T_1 values determined for 7β-acetoxyhopan-22-ol (1b) and 2α,3β,22α-triacetoxystictane (2q) appear in Table 5; a complete list of the ^{13}C T_1 values of these compounds has been included in the supplementary material. We find that in (1b) the T_1 value of C25 (1·35 s) is significantly shorter than that determined for C26 (1·75 s), C27 (1·76 s) or C28 (1·61 s). Amongst these carbons only C25 experiences two 1,3-diaxial methyl–methyl interactions. It is notable that in (2q) the T_1 value of C25 (0·32 s) (the 10β-methyl group carbon) is substantially shorter than that of the corresponding carbon in (1b) (1·35 s). In the stictane skeleton there exists a strong interaction between H9β and the 10β-methyl group protons. By constrast in the hopane skeleton this interaction is replaced by a 1,3-diaxial interaction with the 8β-methyl group protons. It appears that in the former skeleton the H9β interaction is responsible for the

[21] Neto, F. R. A., and Sanders, J. K., *J. Chem. Soc., Perkin Trans. 1*, 1983, 181; Wazeer, M. I. M., *Magn. Reson. Chem.*, 1985, **23**, 249.

Table 4. ^{13}C and ^{1}H n.m.r. assignments (ppm) determined for 2α,3β,22α-triacetoxystictane (2q)

	^{13}C	$^{1}H\alpha$	$^{1}H\beta$		^{13}C	$^{1}H\alpha^{A}$	$^{1}H\beta$
C1	39·0	1·44 (*ax*)	1·81 (*eq*)	C19	34·8	1·05 (*ax*)	1·46 (*eq*)
C2	71·8		5·15 (*ax*)	C20	34·8	1·28 (*eq*)	1·56 (*ax*)
C3	81·0	4·72 (*ax*)		C21	35·2		
C4	38·7			C22	78·3		4·68 (*ax*)
C5	47·6	1·60 (*ax*)		C23	29·4	0·89 (4α-Me)	
C6	18·7	1·16 (*eq*)	1·53 (*ax*)	C24	17·9	0·89 (4β-Me)	
C7	34·0	1·10 (*eq*)	1·93 (*ax*)	C25	23·6	1·03 (10β-Me)	
C8	41·7			C26	22·4	1·14 (8α-Me)	
C9	46·1		1·36 (*ax*)	C27	17·0	0·89 (14β-Me)	
C10	37·3			C28	13·3	0·80 (18β-Me)	
C11	22·7	1·27 (*ax*)	1·50 (*eq*)	C29	29·4	0·84 (21β-Me)	
C12	21·4	1·59 (*eq*)	1·24 (*ax*)	C30	19·5	0·93 (21α-Me)	
C13	48·6	1·32 (*ax*)		$OCOCH_3$	20·8	2·03 (OAc)	
C14	42·5			$OCOCH_3$	20·8	2·03 (OAc)	
C15	31·6	1·28 (*ax*)	1·28 (*eq*)	$OCOCH_3$	20·9	1·97 (OAc)	
C16	19·5	1·21 (*eq*)	1·21 (*ax*)	$OCOCH_3$	170·2		
C17	46·5		1·23 (*ax*)	$OCOCH_3$	170·7		
C18	39·0			$OCOCH_3$	170·8		

[A] Or α-Me, β-Me or acetate.

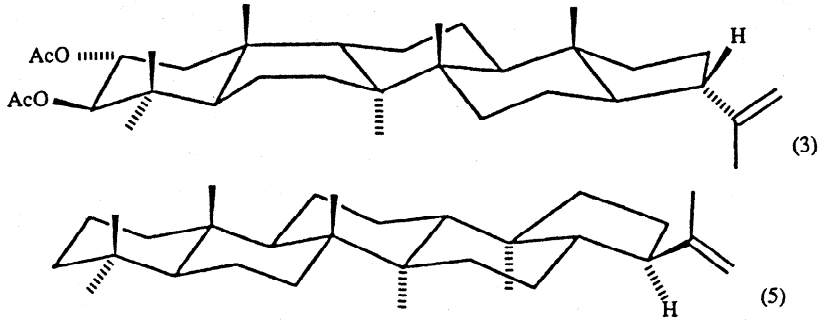
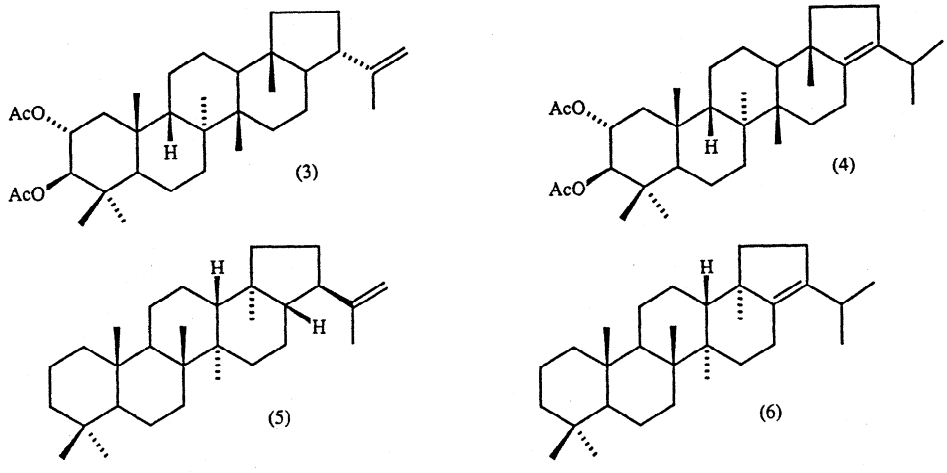


Fig. 4. Three-dimensional conformations of 2α,3β-diacetoxyflavic-22(29)-ene (3) and 21αH-hop-22(29)-ene (5).



marked reduction in the T_1 of C25 and in consequence its T_1 is more akin to that of the equatorial 4α- and 21β-methyl groups.

Table 5. Methyl carbon T_1 values (in s) determined for 7β-acetoxyhopan-22-ol (1b) and 2α,3β,22α-triacetoxystictane (2q)

Error in last significant figure in parentheses.

	(1b)	(2q)		(1b)	(2q)
C23	A	0·35(1)	C27	1·76(5)	1·63(7)
C24	0·97(2)	0·85(3)	C28	1·61(5)	1·42(4)
C25	1·35(3)	0·32(2)	C29	0·44(1)	0·35(1)
C26	1·75(3)	1·56(8)	C30	0·44(1)	A

[A] Coincident with another carbon, T_1 not calculated.

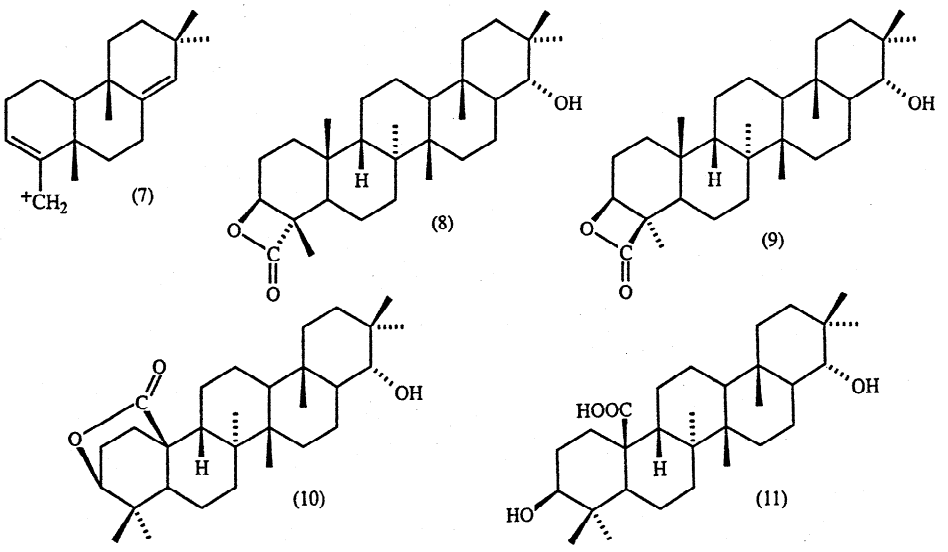
A New Triterpene Lactone

A minor constituent of the extractives of the lichen *P. coronata* was established by high resolution mass spectroscopy to analyse for $C_{30}H_{48}O_3$. The ^{13}C n.m.r. spectrum of the new substance included signals attributable to the ring C/D/E carbons of a 22α-hydroxylated stictane triterpene, together with signals assignable to the oxygenated carbons of a lactone group [178·7 (CO) and 82·7 ppm (–CHOCO–)], while the ^1H n.m.r. spectrum included a signal (4·04 ppm) assignable to the methine proton of a secondary lactone, together with signals from seven rather than the usual eight methyl groups encountered in stictane triterpenoids. An ion of *m/z* 257 (2%) ($C_{19}H_{29}$ by high resolution measurement), for which structure (7) has been proposed,[22] appeared in the mass spectrum of the new compound; hence it can be reasoned that the 8α-, 14α-, 18α-, 21α- and 21β-methyl groups have not been functionalized. On the other hand biosynthetic considerations lead to the conclusion that an oxygen functionality should be present at C3 since 2,3-epoxysqualene is considered[2] to be an intermediate in the biosynthesis of stictane triterpenes. On this basis plausible structures for the new triterpene include (8)–(10).

Unequivocal evidence for the functionalization of the 10β-methyl group, as opposed to one of the C4 methyl groups, was obtained in an absolute value double quantum filtered COSY n.m.r. experiment. Since two pairs of methyl group protons (0·86/0·98 and 0·87/1·00 ppm) proved to be mutually ^4J coupled (see Fig. 5) it follows that neither of the C21 and C4 *gem*-dimethyl groups has been functionalized. Accordingly it can be concluded that the new triterpene is 22α-hydroxystictano-25,3β-lactone (10). Presumably biosynthesis of the lactone (10) involves the intermediacy of 3β,22α-dihydroxystictan-25-oic acid (11).

A notable feature of structure (10) is the requirement that ring A must adopt a boat conformation analogous to that obtained on lactonization of *cis*-4-hydroxycyclohexane-1-carboxylic acid[23] and in consequence the $^3J_{(H3\alpha-H2\beta)}$ and $^3J_{(H3\alpha-H2\alpha)}$ couplings determined for 22α-hydroxystictano-25,3β-lactone (9) (*J* 4·1 and 1·4 Hz) differ substantially from those observed at 300 MHz

[22] Holland, P. T., and Wilkins, A. L., *Org. Mass Spectrom.*, 1979, **14**, 160.
[23] Eliel, E. L., 'Stereochemistry of Carbon Compounds' p. 208 (McGraw-Hill: New York 1962).



for H3α of the stictane-3β,22α-diol (2c) (J 10·9 and 4·5 Hz). The ^{1}H n.m.r. spectrum of the lactone (10) is additionally characterized by a one-proton doublet of doublets centred at 2·57 ppm (J 12·3 and 3·3 Hz). This signal can be assigned to H9β since molecular models indicate that the carbonyl group of the lactone (10) is likely to strongly deshield this proton. In the double quantum filtered COSY spectrum this proton exhibited cross peaks at 1·28 and 1·55 ppm; these resonances correspond closely to those determined for H11α

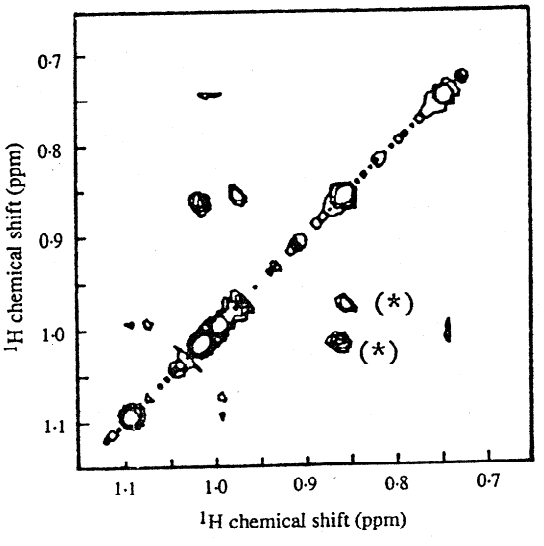


Fig. 5. Absolute value double quantum filtered COSY n.m.r. spectrum of 22α-hydroxystictano-25,3β-lactone (10) (methyl proton region only). Cross peaks attributable to ^4J couplings between the protons of the 4α-, 4β-, 21α- and 21β-methyl groups are marked with an asterisk.

(1·27 ppm) and H11β (1·50 ppm) of the triacetate (2q) (see Table 4). The couplings experienced by H9β [$^3J_{(H9\beta-H11\alpha)}$ = 12·4 Hz and $^3J_{(H9\beta-H11\beta)}$ = 3·3 Hz] can therefore be assigned. Finally the double boat conformation required for (10) can also be compared to that adopted in the solution state[2] and in the solid state[19] by 22α-hydroxystictan-3-one (2g).

Experimental

The isolation, structural elucidation and/or syntheses of the stictane, flavicene and hopane triterpenes, other than (2p), have been reported previously.[1-3] ^{1}H and ^{13}C n.m.r. spectral data presented in Tables 1, 4, and 5 were recorded on a Varian XL-300 spectrometer. The ^{13}C n.m.r. spectra of other compounds (Tables 2 and 3) were recorded on a Jeol FX-90Q spectrometer except for the spectrum of (2l) which was determined on a Bruker AC-200 spectrometer. Chemical shifts were determined in $CDCl_3$ and are reported relative to internal $CDCl_3$ (δ 77·1 ppm). Discrepancies, where they exist, between the ^{1}H n.m.r. data reported here and in the literature cited are not considered significant given the established[8] concentration dependence of chemical shift data. The LIS study was performed with Eu(dpm)$_3$ as shift reagent. 2D n.m.r. and T_1 acquisition and processing conditions are given in the supplementary material (see below).

2α,3β-Diacetoxystictan-22β-ol (2p)

Sodium borohydride (100 mg) was added to a stirred solution of 2α,3β-diacetoxystictan-22-one (2o) (160 mg) in dioxan–methanol–water (4 : 4 : 1) (80 ml). After 30 min the excess borohydride was destroyed by the addition of 2 M hydrochloric acid, and the reaction mixture worked up in the usual way.[1] Purification by preparative layer chromatography (p.l.c.) on silica gel with ether–hexane (2 : 3) as eluent afforded *2α,3β-diacetoxystictan-22β-ol* (2p), m.p. 236–241° (from acetone) (Found: C, 74·8; H, 10·7. $C_{34}H_{56}O_5$ requires C, 75·0; H, 10·4%). ν_{max} (KBr) 3510 (OH), 1763, 1724 (OAc) cm^{-1}. ^{1}H n.m.r. δ (90 MHz, $CDCl_3$) 0·92, s, 3xCH_3; 0·95, 0·99, s, CH_3; 1·04, s, CH_3; 1·17, s, CH_3; 1·98, s, $OCOCH_3$; 2·05, s, $OCOCH_3$; 3·17,m, $W_{h/2}$ 4·5 Hz, H22α; 4·72, d, J 10 Hz, H3α; 5·17, sextet, J 10, 10, 5 Hz, H2β.

Isolation of 22α-Hydroxystictano-25,3β-lactone (10)

Separation of the hexane extracts (1·2 g) of *P. coronata* (45 g) by multiple p.l.c. on silica gel, as previously reported,[3] afforded a band of R_F value intermediate between that of 22α-hydroxystictan-3-one (2g) and 2α,3β-diacetoxystictan-22α-ol (2j). Crystallization from chloroform–hexane afforded *22α-hydroxystictano-25,3β-lactone* (10) (4 mg), m.p. 320–324° (Found: M$^{+\bullet}$ 456·3640. $C_{30}H_{48}O_3$ requires M$^{+\bullet}$ 456·3605). ν_{max} (Nujol) 3420 (OH), 1740 (CO), 1136, 1060 cm^{-1}. ^{1}H n.m.r. δ (300 MHz, $CDCl_3$) 0·76, s, CH_3; 0·86, s, CH_3; 0·87, s, CH_3; 0·98, s, CH_3; 1·00, s, CH_3; 1·03, s, CH_3; 1·10, s, CH_3; 2·57, dd, J 12·3, 3·3 Hz, H9β; 3·16, d, J 11·1 Hz, H22β; 4·04, dd, J 4·1, 1·4 Hz, H3α. ^{13}C n.m.r.: see Table 2. Mass spectrum (70 eV): *m/z* 456 (M$^+$, 3%), 438 (26), 423 (13), 395 (15), 369 (11), 293 (66), 225 (11), 221 (16), 207 (40), 205 (30), 204 (22), 203 (20), 201 (11), 191 (48), 190 (43), 189 (100), 188 (15), 187 (27). [Found for ion (7) *m/z* 257·2251. $C_{19}H_{29}$ requires *m/z* 257·2271.]

Deposited Material

2D n.m.r. and T_1 acquisition and processing conditions, coupled and decoupled PSHCOR spectra of (1b) including proton multiplicity cross section profiles, resolution enhanced conventional ^{1}H n.m.r. spectrum showing methyl group ^4J couplings, and tabulations of ^{1}H and ^{13}C n.m.r. data (chemical shifts, T_1 values, line widths, etc.) for (1b) and (2q) are available from the Australian Journal of Chemistry, 314 Albert Street, East Melbourne, Vic. 3002.

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